Review Article

Monitoring therapeutic response and resistance with liquid biopsy

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Abstract: Lung cancer is the leading cause of cancer-related death in industrialized countries and one of the most common cancers in the world. The most common type of lung cancer is non-small cell lung cancer (NSCLC), which accounts for 80–90%. Approximately 3–7% of NSCLC patients have a genomic rearrangement of the anaplastic lymphoma kinase (*ALK*) gene. Tyrosine kinase inhibitors (TKIs) of the *ALK* have significantly improved the quality of life and survival of *ALK*-positive NSCLC. The therapeutic arsenal includes first-generation ALK-TKI: crizotinib and more recently second-generation ALK-TKI: ceritinib, ensartinib, alectinib and brigatinib and third-generation ALK-TKI: lorlatinib. Second-generation ALK-TKIs have become the new standard of care in first-line setting in advanced *ALK*-positive NSCLC patients. However, most of these patients relapse as the tumor acquires resistance mutations. Although the rebiopsy at the time of ALK-TKI progression is not always feasible, liquid biopsy at progression is a potential alternative tool. Circulating tumor cells (CTCs), circulating free tumor DNA (cfDNA), exosomes and tumor-educated platelets (TEPs) in body fluid could be used to monitor response to treatments and resistance mechanisms, this may provide relevant information to define genomic-driven therapeutic sequences. Even though, the use of ALK-TKI according to the resistance mechanism, at the time of progression, seems the most appropriate, blinded treatment decisions are the most common in the clinic.

Keywords: Anaplastic lymphoma kinase (*ALK*); liquid biopsy; next-generation sequencing (NGS); non-small cell lung cancer (NSCLC); resistance

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Introduction

Lung cancer is the leading cause of cancer-related death in industrialized countries and one of the most common cancers in the world. A total of 29,638 new cases estimated per year in Spain in the general population in 2020 and 22,153 deaths in 2019. The most common type of lung cancer is non-small cell lung cancer (NSCLC), which accounts for 80–90%. Approximately 3–7% of NSCLC patients have a genomic rearrangement of the anaplastic lymphoma kinase (*ALK*) gene (1).

ALK is a tyrosine kinase encoded on chromosome 2 and is primarily involved in developmental processes and expressed at low levels in adults. The first and the most prevalent fusion partner involved a fusion between the echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the *ALK* tyrosine kinase domain (2). Other additional *ALK* fusion partners have been described in NSCLC that are believed to result in aberrant signaling and oncogenic transformation. *ALK* rearrangements are more

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common among patients with adenocarcinoma histology, patients who have never smoked, and patients who have wild-type *EGFR* and *KRAS*.

The standard treatment algorithm for unselected NSCLC patients has historically involved front-line treatment with chemotherapy, however recent clinical studies have demonstrated that patients with *ALK*-positive locally advanced or metastatic NSCLC respond well to treatment with an ALK tyrosine kinase inhibitor (ALK-TKI), it is an effective targeted medicine for these patients and can significantly prolong their survival.

ALK-TKI

The therapeutic arsenal includes first-generation ALK-TKI: crizotinib and more recently second-generation ALK-TKI: ceritinib, ensartinib, alectinib and brigatinib and third-generation ALK-TKI: lorlatinib.

Crizotinib is a first-generation ALK-TKI. It is a selective small molecule inhibitor of ALK, hepatocyte growth factor receptor (HGFR), c-MET and ROS-1. In preclinical studies, crizotinib demonstrated a concentration-dependent inhibitory activity of the growth of cell lines and animal models (xenografts) carrying the ALK, c-MET and ROS-1, inducing a powerful cellular apoptosis of the same, what was correlated, from a pharmacodynamic point of view, with the inhibition of *ALK* fusion protein phosphorylation in tumors *in vivo*. It has been used as a first-line therapy for patients with *ALK*-positive locally advanced or metastatic NSCLC, demonstrating objective response rate (ORR) around 60–70%, progression-free survival (PFS) around 8–11 months and specific improvements in quality of life with respect to treatments according to the results of the main phase III studies, PROFILE 1007 (3) and PROFILE 1014 (4,5).

PROFILE 1007 is a phase III, open-label, randomized clinical trial that evaluates the efficacy and safety of crizotinib for the treatment of patients with *ALK*-positive metastatic NSCLC, who had received previous systemic treatment for advanced disease versus standard of care. The full analysis population included 347 patients with *ALK*-positive advanced NSCLC as identified by FISH prior to randomization. One hundred seventy-three patients were randomized to crizotinib and 174 patients were randomized to chemotherapy (either pemetrexed or docetaxel). Crizotinib significantly prolonged PFS, the primary objective of the study, compared to chemotherapy as assessed by IRR. The median PFS was 7.7 months for the group treated with crizotinib versus 3.0 months for the group treated with chemotherapy. The PFS benefit of crizotinib was consistent across subgroups of baseline patient characteristics such as age, gender, race, smoking class, time since diagnosis, ECOG performance status, presence of brain metastases and prior EGFR TKI therapy. There was a numerical, but not statistically significant, improvement in overall survival (OS) for crizotinib *vs.* chemotherapy. The crossover was allowed.

In the PROFILE 1014 clinical trial it was evaluated the efficacy and safety of crizotinib for the treatment of patients with *ALK*-positive metastatic NSCLC, who had not received previous systemic treatment for advanced disease. The full analysis population included 343 patients with *ALK*-positive advanced NSCLC as identified by fluorescence in situ hybridization (FISH) prior to randomization: 172 patients were randomized to crizotinib and 171 patients were randomized to chemotherapy, 6 cycles of platinum doublet (pemetrexed + carboplatin or cisplatin). PFS was significantly longer with crizotinib, 10.9 months, than with chemotherapy, 7.0 months. ORR were 74% and 45%, respectively (P<0.001).

The problem is that about 30% of patients who used crizotinib as initial treatment have primary resistance to it and some patients developed secondary resistance within 1–2 years. Further, about 40% developed metastasis in central nervous system (CNS) (6).

Other ALK-TKI were gradually introduced and approved for clinical application.

Ceritinib is a potent oral, ATP-competitive inhibitor of the tyrosine kinase domain of *ALK*. In addition, it inhibits insulin-like growth factor receptor 1 (IGF-1R), the insulin receptor (InsR) and ROS1. Ceritinib is 20 times more potent than crizotinib. Phase I trial demonstrated that ceritinib induced responses in up to 60% of ALK-positive NSCLC patients. Ceritinib is equally active in patients previously treated with crizotinib as in those who have not received this treatment. Likewise, it shows response in brain metastases in patients who have progressed to crizotinib. On the other hand, ceritinib appears to be active regardless of the acquired resistance mechanism. The FDA in 2014 granted accelerated approval of ceritinib as a treatment for patients diagnosed with advanced ALK-positive advanced NSCLC in a state of progression after treatment with crizotinib or in cases of intolerance to crizotinib. The phase III study, ASCEND-4 (7) compared ceritinib 750 mg/day versus platinum-based chemotherapy (cisplatin 75 mg/m^2 or carboplatin AUC 5–6 plus pemetrexed 500 mg/m^2) every 3 weeks for four cycles followed by maintenance

pemetrexed in patients with *ALK*-positive advanced NSCLC not previously treated. The ORR for patients who received ceritinib was 72.5% compared to 26.7% for the group of patients who received chemotherapy, and the PFS was statistically higher also for the group of patients who received ceritinib 16.6 months versus 8.1 months for patients who received chemotherapy (HR 0.55, 95% CI: 0.42–0.73, P<0.00001). The intracranial ORR determined by RECIST v1.1 was 72.7% with ceritinib *vs.* 27.3% with chemotherapy, while the PFS in patients without basal brain metastases was 26.3 months with ceritinib versus 8.3 months with chemotherapy.

Alectinib is a highly selective inhibitor of ALK and has shown systemic and CNS superiority in the first line compared to crizotinib in two phase III studies. The J-ALEX study (8), conducted exclusively in Japan, included 207 patients with advanced *ALK*-positive NSCLC, who were chemotherapy-naïve or had received one previous chemotherapy regimen. This study confirmed a significant benefit in terms of PFS for alectinib 300 mg/12 hours (HR: 0.34; P<0.0001) compared to crizotinib. Recently it has been reported final PFS data and the second pre-planned interim analysis of OS and safety (9). Median follow-up was 42.4 months for alectinib and 42.2 months for crizotinib. At the final PFS analysis, alectinib continued to demonstrate superiority in independent review facility (IRF)-assessed PFS versus crizotinib in ALK-inhibitor-naïve *ALK*-positive NSCLC (HR 0.37, 95% CI: 0.26–0.52; median PFS 34.1 *vs.* 10.2 months crizotinib), with a favorable safety profile. At the second interim OS analysis, superiority of alectinib to crizotinib could not be concluded (stratified HR 0.80, 99.8799% CI: 0.35–1.82, stratified log-rank P=0.3860; median OS not reached alectinib *vs.* 43.7 months crizotinib). OS follow-up continues. The global phase III study ALEX (10), conducted in 303 patients with advanced *ALK*-positive NSCLC not previously treated, compared alectinib 600 mg/12 hours *vs.* crizotinib 250 mg/12 hours. With a follow-up of 18.6 months for alectinib and 17.6 months for crizotinib, the PFS evaluated by an independent radiological committee (IRC) was 25.7 *vs.* 10.4 months (HR 0.50, P<0.001). An update of the results of this study, with an additional follow-up, confirmed the benefit in PFS documented by the investigator (34.8 *vs.* 10.9 months, HR 0.43, and ORR 82.9 *vs.* 75.5%) for alectinib compared to crizotinib (11). OS data is still immature (37% of events). Median OS was not reached with alectinib versus 57.4 months with crizotinib (stratified HR 0.67, 95% CI: 0.46–0.98). The 5-year OS rate was 62.5% (95% CI: 54.3–70.8%) with alectinib and 45.5% (95% CI: 33.6–57.4%) with crizotinib, with 34.9% and 8.6% of patients still on study treatment, respectively. The OS benefit of alectinib was seen in patients with CNS metastases at baseline and those without (12).

Brigatinib is a TKI that targets *ALK*, *c-ros* oncogene 1 (*ROS1*) and insulin like growth factor 1 receptor (IGF 1R). Brigatinib inhibits autophosphorylation of ALK and ALK mediated phosphorylation of the downstream signaling protein STAT3 in vitro and in vivo assays.

The efficacy and safety assessment of brigatinib is based on three studies:

The study AP26113-11-101 (13) was a phase I/II study of the safety, tolerability, pharmacokinetics (PK) and preliminary antitumor activity of brigatinib in advanced malignancies, including *ALK*-positive NSCLC. A total of 137 patients with advanced cancer were enrolled, including 79 patients (58%) with *ALK*-positive NSCLC, of which 71 had been previously treated with crizotinib. A total of 50 *ALK*-positive NSCLC patients had brain metastases at baseline. The 90mg/180mg cohort included 25 patients with *ALK*-positive NSCLC previously treated with crizotinib. In this group, 19/25 patients (76.0%; 95% CI: 54.9–90.6%) had a confirmed objective response. The median time to response was 1.9 months (range, 1.2–6.0 months). The median PFS was 16.3 months (95% CI: 9.2–not reached). The median OS was not reached in this group. Of those patients with *ALK*-positive NSCLC previously treated with crizotinib in the 90/180 mg group, there were 18 evaluable patients with brain metastases at baseline, of which 8 (44.4%; 95% CI: 21.5–69.2%) had a confirmed response by IRC. The median intracranial duration of response was 11.4 months (95% CI: 5.6–11.4 months) and the median intracranial PFS was not reached.

Most crizotinib-treated patients with *ALK*-positive NSCLC eventually experience disease progression.

The study AP26113-13-201 (ALTA) (14) was a pivotal phase II study in patients with advanced ALK-positive NSCLC whose disease had progressed to crizotinib. A total of 222 patients were enrolled and randomized 1:1 to receive brigatinib 90 mg once daily (112 patients) or brigatinib 180 mg once daily with a 7-day lead-in at 90 mg (110 patients). Patients were stratified by CNS metastases and best response to crizotinib. 154 (69%) had baseline brain metastases and 164 (74%) had received prior chemotherapy. The primary end point was investigator-assessed confirmed ORR per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Secondary end points included

independent review committee (IRC)-assessed PFS, intracranial PFS (iPFS) and OS. The median follow-up was 19.6 versus 24.3 months. Investigator-assessed confirmed ORR was 45.5% (97.5% CI: 34.8–56.5%) for the 90 mg group compared to 56.4% (97.5% CI: 45.2–67.0%) for the 90/180 mg group. Confirmed CR by investigator assessment was also increased for the 90/180 mg group: 4.5% *vs.* 1.8%. The ORR by IRC was similar. The median time to response was 1.8 to 1.9 months. The updated estimate median investigator-assessed duration of response was 12.0 months (95% CI: 9.2–17.7 months) for patients in the 90 mg group and 13.8 months (95% CI: 10.2–19.3 months) for patients in 90/180 mg group. IRC assessed duration of response was in line. Median IRC-assessed PFS was 9.2 months (95% CI: 7.4–12.8 months) for the 90 mg group and 16.7 months (95% CI: 11.6–21.4 months) for the 90/180 mg group. PFS results assessed by investigators were also similar. Median OS was 29.5 months in the 90 mg once daily group (95% CI: 18.2–not reached) versus 34.1 months (95% CI: 27.7–not reached) in the 90/180 mg group. Brigatinib showed promising efficacy in the CNS, IRC-confirmed intracranial ORR in patients with measurable baseline brain lesions was 50% (13 of 26) versus 67% (12 of 18); median duration of intracranial response was 9.4 versus 16.6 months. IRC-assessed iPFS was 12.8 versus 18.4 months (15).

The third study that evaluated the role of brigatinib was the AP26113-13-301 (ALTA-1L) (16), an open-label, phase III trial, comparing brigatinib 180 mg once daily, after 7-day lead-in period at 90 mg once daily, or crizotinib at a dose of 250 mg twice daily. The primary end point was PFS as assessed by blinded independent central review. Secondary end points included the ORR and intracranial response. A total of 275 patients, not previously treated with ALK-TKI but who may be chemotherapy-naïve or who may have received one previous chemotherapy regimen, were randomized. Recently it has been published the second interim analysis (150 events) (17), the median follow-up was 24.9 months in the brigatinib arm and months in the crizotinib arm. Brigatinib showed consistent superiority in BIRC-assessed PFS versus crizotinib (HR 0.49, 95% CI: 0.35–0.68; log-rank P<0.0001; median, 24.0 *vs.* 11.0 months, respectively). Investigator-assessed PFS HR was 0.43 (95% CI: 0.31–0.61), median PFS was 29.4 *vs.* 9.2 months. Brigatinib significantly delayed both CNS progression (without prior systemic progression) and systemic progression (without prior intracranial progression) compared to crizotinib.

Currently, two other phase III clinical trials are evaluating the value of lorlatinib (CROWN, NCT03052608) and ensartinib (eXalt3, NCT02767804) compared to crizotinib in patients with advanced *ALK*-positive NSCLC. In the phase III CROWN study compared lorlatinib and crizotinib in first-line treatment in 296 *ALK*-naïve NSCLC patients. Crossover was not allowed. PFS by BICR (blinded independent review committee) was significantly prolonged with lorlatinib *vs.* crizotinib (HR 0.28; 95% CI: 0.19–0.41; P<0.001, not reached median PFS with lorlatinib *vs.* 9.3 months with crizotinib). Lorlatinib also improved RR by BIRC compared with crizotinib (76% *vs.* 58%, respectively) and intracranial activity RR among patients with measurable brain metastases (82% *vs.* 23%) (18).

Positive findings from the eXalt3 trial of ensartinib have been reported at the 2020 Virtual Presidential Symposium of the World Congress on Lung Cancer. Ensartinib is an ALK-TKI, with potency 10 times greater than crizotinib in enzymatic assays and broad preclinical activity in *ALK* resistance mutations. In a phase II trial ensartinib had activity and was well tolerated in patients with crizotinib-refractory, ALK-positive NSCLC, including those with brain metastases (19). The primary endpoint of the phase III trial of BIRC-assessed PFS in the ITT population was 25.8 months for the patients assigned to receive ensartinib versus 12.7 months for the patients who received crizotinib. OS is still immature. Ensartinib showed a favourable safety profile, serious treatment-related adverse events (TRAEs) occurred in 8% of the patients treated with ensartinib and 6% of those who received crizotinib.

Although crizotinib, ceritinib, alectinib and brigatinib are recommend in the first line setting by the NCCN guidelines, alectinib is considered the preferred option.

ALK-TKI resistance

In approximately 50% of patients the CNS is the first site of progression (20), suggesting inadequate penetration of crizotinib into the brain (i.e., pharmacologic failure) as the primary cause of resistance in these patients. Intra-tyrosine kinase secondary *ALK* mutations represents the main mechanism of resistance to second-generation ALK TKIs, reported in more than 50% of patients. The same patient may have two or more resistance patterns.

Although, a large part of the resistance mechanism still remains unknown, ALK-TKI resistance can be divided into primary resistance and secondary resistance.

Primary resistance or intrinsic resistance refers to the ineffective treatment from the start. About 25% of

patients treated with crizotinib have primary resistance. It is speculated that the mechanism of primary resistance is closely related to *ALK* variations, such as pre-existing *ALK* polymorphisms, deletions, and mutations (21). Tumor heterogeneity can also lead to primary resistance. Studies have found that 5–8% of cancer cells in *ALK*-positive NSCLC patient contain *EGFR* mutation, which causes the failure of ALK-TKI. Another cause may be false-positive genotype.

Secondary resistance or acquired resistance refers to the recurrence of tumor or tumor progression after having achieved complete response or partial response to ALK-TKI (22). Secondary resistance can be divided into dominant and non-dominant.

Dominant secondary resistance mainly refers to *ALK* kinase domain mutation (29%) and increase of *ALK* gene copy number (9%), which accounts for one-third of crizotinib resistance by increasing the activity of tyrosine kinases. Most of the mutations currently found in the target *ALK* gene are mainly point mutation. C1156Y and L1196M were the first discovered mutant types, followed by L1152R, G1202R, G1269A, F1174L, 1151Tins, S1206Y, I1171T, D1203N, V1180L, etc. (23-26). The most common mutation of *ALK* kinase domain in the crizotinib resistance is L1196M and G1269A (27). Patients with L1196M are sensitive to treatment with second-generation ALK-TKI.

ALK resistance mutations were different after crizotinib or after treatment with second-generation ALK-TKI. *ALK* resistance mutations were present in over one-half of patients progressing to second-generation ALK-TKI and also the spectrum of *ALK* resistance mutations was different following progression on second-generation ALK-TKI compared to crizotinib. Most notably, ALK G1202R, which was present in only 2% of crizotinib-resistant biopsies, emerged as the most common *ALK* resistance mutation after treatment with second-generation ALK-TKI. While ALK G1202R was a common shared resistance mutation in each second-generation ALK, it is noteworthy that the spectrum of other *ALK* resistance mutations appeared to different across agents. For example, *ALK* mutations such as F1174L/C, C1156Y and L1196M were observed in several post-ceritinib biopsy specimens, alectinib also included I1171T/N/S, V1180L and L1196M while E1210K, D1203N and S1206Y/C ALK mutations appears to be more frequent with brigatinib (24).

Non-dominant secondary resistance includes ALK signal bypass activation, and tumor heterogeneity. Signal bypass activation refers to the activation of other carcinogenic drivers and results in 20% of the cases of crizotinib resistance (28). The most common driven genes include *EGFR* and *KRAS* mutations, *EGFR* phosphorylation and *c-KIT* amplification. Other mechanisms is transformation to small cell lung cancer (SCLC), it involves tumor suppressor genes such as, *Rb1* and *p53*, and this has been observed in a patient with crizotinib treatment failure (29) and in a patient with alectinib treatment failure (30).

Liquid biopsy

At present, the follow-up of patients with lung cancer during treatment mainly depends on the imaging tests and pathological biopsy. However, pathological biopsy is an invasive test and is not always possible to perform. Liquid biopsy emerges as a useful tool to provide genetic landscape of cancer lesions (31). The most common biomarkers of liquid biopsy are circulating tumor cells (CTCs), circulating free DNA (cfDNA), exosomal RNA or tumor platelets and they offer the potential for early diagnosis, identification of therapeutic targets, real-time monitoring of therapies and resistance mechanisms. Therefore, liquid biopsy might be the most convenient in the clinical setting particularly when tissue is unavailable or as a first screening for assessing resistance. It has different advantages such as simplicity, minimally invasive, real time and reproducibility.

The CTCs are the tumor cells that fall off from primary lesions or metastases of tumor ad then enter into the blood circulation (31). The detection rate of CTCs is low; only one CTC can be detected in an average of 10^5–10^7 monocytes in the peripheral blood of patients with advanced tumors (32). There are several methodologies for CTC detection, counting and characterization. The CellSearch system which is approved by FDA. In addition, Digital droplet polymerase chain reaction combined with next-generation sequencing (NGS) is currently used to detect and sequence genes in CTCs. CTCs presence and counting has been associated with worse prognosis in *ALK*-positive NSCLC patients (33). Moreover, some researchers have reported that ALK status can be effectively assessed in CTCs isolated from NSCLC patients (34,35). However, sample sizes of the aforementioned studies are rather small and results from larger cohort are needed in order to determine the clinical utility of this approach in NSCLC patients harboring an *ALK* translocation.

Circulating free DNA (cfDNA) is defined as the tissue-specific DNA fragment that is released into the blood (36).

Circulating tumor DNA (ctDNA) refers to the cfDNA that is secreted by tumor cells or released after apoptosis and necrosis (37). The ctDNA levels in cancer patients are positively correlated with tumor progression, and the half-life is generally about 2 hours. While ctDNA has been widely used for the detection of *EGFR* activating mutations in NSCLC patients (38-40), less progress has been made regarding non-invasive ALK testing. Nonetheless, *ALK* rearrangements can be detected through ctDNA analysis (41,42).

Noteworthy, in a cohort of 88 consecutive patients with 96 plasma-detected ALK fusions McCoach *et al*. demonstrate that comprehensive cfDNA NGS (Guardant360) provides a noninvasive means of detecting *ALK* fusions and characterizing resistance mechanism on progression (43). Nevertheless, *ALK* rearrangements are difficult to detect since these alterations involve a large number of base pairs and cfDNA is typically fragmented. In addition, breakpoints at DNA levels are different in each case. Therefore a negative result should be taken with caution. Conversely, point mutations in *ALK* locus are easier to detect. In this way the detection of resistance mutations in *ALK* locus, upon ALK-TKI failure is of particular interest in order to optimize the sequencing of ALK-TKI maximizing patient's survival. Several cohorts describing the resistance mechanisms ALK-TKI using liquid biopsies have been described (44,45). The clinical utility of longitudinal plasma genotyping in monitoring the evolution of *ALK*-positive NSCLC patients have been also evaluated (46). In this way, our group has recently published a case report where we illustrate the usefulness of plasma NGS profiling for detecting drug resistance mechanism upon disease progression in NSCLC patients with *EML4-ALK* rearrangement (47).

On the other hand, disease burden has been correlated not only with the appearance of resistance mutations but with the quantitative values of the allelic frequency (AF) of plasma alterations (48). Similarly, it has been recently reported that ctDNA quantification is of prognostic significance, in terms of PFS, in *ALK*-positive patients (49).

Exosomes are extracellular vesicles (EVs) with a diameter of 30-120nm, the surface of which is made up of lipid bilayer vesicles, containing DNA, mRNA, miRNA, protein and other genetic materials (50). Exosomes have important implications in resistance testing, and it may be more valuable than the CTCs and the ctDNA. The concentration of exosomes in peripheral blood is higher than that of CTCs, and it can be detected in urine, saliva, cerebrospinal fluid, semen, milk, pleural effusion, ascites and other body fluids in addition to serum or plasma (51). Exosomes arose as a promising tool for *ALK* fusion testing since fusion detection at the RNA level is feasible. Of note, RNA inside EVs is protected from degradation and therefore is susceptible of being subjected to analysis. Reclusa *et al*. recently reported the identification of *EML4-ALK* translocation exosomal RNA derived from NSCLC patients (52). In this study only 19 patients with plasma samples were included (16 of them harboring an *ALK* fusion) but it opens the door for the development of non-invasive *ALK* fusion testing strategies based on EVs isolation.

Finally, tumor-educated platelets (TEPs) are emerging as promising biomarker source. Tumors are known to educate platelets by altering its RNA profile. The analysis RNA obtained from TEPs has been demonstrated to detect early- and late-stage NSCLC (53). TEPs may offer certain advantages over other non-invasive analytes, including their abundance and easy isolation, high-quality RNA obtained from them. It has been reported that non-invasive *ALK* testing can be performed using TEPs (53,54). However, larger studies are needed to further support the use of TEPs in the management of *ALK*-positive NSCLC patients.

Other body fluids like urine, saliva and pleural effusion have been used on the diagnosis of NSCLC. There are many studies on EGFR-TKI resistance surveillance, however, more studies and experiments are expected to confirm their viability in ALK-TKI resistance surveillance.

Discussion and conclusions

Liquid biopsy is applicable to the surveillance of all lung cancer patients, can predict tumor progression and plays an important role in warning resistance. This can help us prolong the survival and avoid side effects caused by ineffective treatment. In advanced *ALK*-positive NSCLC, alectinib has shown the best balance of efficacy and safety and is the current preferred first-line treatment option. Lorlatinib is approved in advanced *ALK*-positive NSCLC patients whose disease has progressed to prior second-generation ALK TKIs. It is very interesting to known the molecular profiling at the time of disease progression to second and third-generation ALK TKIs because this may provide relevant information to define genomic-driven therapeutic sequences. Even though, the use of ALK-

TKI according to the resistance mechanism, at the time of progression, seems the most appropriate, blinded treatment decisions are the most common in the clinic.

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